

# Novel Sylvatic Rabies Virus Variant in Endangered Golden Palm Civet, Sri Lanka

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Information is scarce about sylvatic rabies virus in Asia and about rabies in palm civets. We report a novel sylvatic rabies virus variant detected in a golden palm civet in Sri Lanka. Evolutionary analysis suggests the virus diverged from canine rabies viruses in Sri Lanka in  $\approx 1933$  (range 1886–1963).

Rabies has been eliminated from domestic animals in industrialized countries, but sylvatic rabies remains an endemic disease. The ecology of rabies in wildlife populations and natural ecosystems is poorly understood (1), and, as a result, eliminating rabies from the wild is difficult. Little is known about sylvatic rabies in developing countries, where rabies takes its biggest toll on humans. Rabies is endemic to Sri Lanka and has been identified in different wild animals. However, all documented cases of rabies in wildlife in Sri Lanka have been considered a consequence of spillover from dogs. Rabies viruses circulating in this country are distinctly highly homogeneous (2,3).

Two species of palm civet are commonly found in Sri Lanka: the common palm civet, *Paradoxurus hermaphroditus*, which is widespread in southern Asia and Southeast Asia, and the golden palm civet, *P. zeylonensis*, which is indigenous to Sri Lanka. This species is closely related to the brown palm civet (*P. jerdoni*), which lives only in southern India (4). Moreover, 3 additional new species have been identified in Sri Lanka: the golden wet-zone palm civet (*P. aureus*), the golden dry-zone palm civet (*P. stenocephalus*), and the Sri Lankan brown palm civet

(*P. montanus*) (4). Palm civets in Sri Lanka are, however, endangered because of hunting, parasitic diseases, and dwindling habitat. We report on a sylvatic rabies virus variant detected in a golden palm civet in Sri Lanka.

## The Study

On a November morning in 2009, a “wild cat” appeared in the garden of a basic health clinic in Moneragala district, Uva Province, Sri Lanka. The animal, which showed aggressive behavior, was suspected to be rabid and was thus killed to prevent transmission of rabies virus to humans. The animal’s head was packed in ice to avoid decomposition and sent to the Medical Research Institute (Colombo, Sri Lanka) for testing. We detected rabies virus in the animal’s brain by using the fluorescent antibody test and extracted viral RNA and DNA by using Trizol (Invitrogen, Carlsbad, CA, USA). The rabies virus from this sample was designated as H-1413-09. The whole genome of the virus was sequenced directly from the sample as described (5).

To confirm the species of the rabid animal, we determined the nucleotide sequence of the mitochondrial cytochrome *b* (*cytb*) gene and performed a BLAST search ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) for similarity with other sequences. By aligning nucleotide sequences of the *cytb* gene of mitochondrial DNA of domestic cat, jungle cat, fishing cat, Asiatic golden cat, marbled pole cat, European pole cat, lynx, puma, leopard, African lion, tiger, jaguar, civet, and palm civet with ClustalW2 ([www.ebi.ac.uk/clustalw](http://www.ebi.ac.uk/clustalw)), we designed primer Felis *cytb*-F, 5'-ATGACCAACATTTCGAAAATCACACC-3' (nt 1–25), and primer Felis *cytb*-R, 5'-CAATAATGCCTGAGATGGGTATTAG-3' (nt 1093–1,117). Using these primers, we performed PCR as follows: initial denaturation at 94°C for 2 min, followed by 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min for 35 cycles, followed by a final extension at 72°C for 5 min. PCR generated a 1,117-bp fragment from which a 1,004-nt sequence was determined. Analysis showed that the sequence has 100% identity with the partial (224-nt) sequence of the *cytb* gene of *P. zeylonensis* (GenBank accession no. FJ881681); this is the only sequence available for *P. zeylonensis*. The sequence also has 95% identity with *P. jerdoni* and 90%–92% identity with *P. hermaphroditus*.

We performed an evolutionary analysis by using the N gene. We inferred a maximum clade credibility phylogenetic tree by using the Bayesian Markov chain Monte Carlo method available in BEAST version 1.6.1 (6). The analysis used a relaxed (uncorrelated lognormal) molecular clock and GTR +  $\Gamma$  + I model of nucleotide substitution. We selected the model on the basis of Akaike Information Criterion by using jModelTest software (7). All chains were run for  $9 \times 10^7$  generations and sampled

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results support our finding that strain H-1413-09 differs from other rabies viruses circulating in Sri Lanka.

## Conclusions

Rabies virus probably survives favorably in the wild because it can infect a large spectrum of animals, thereby

Table 2. Substitutions in genome sequence of rabies virus strain H-1413-09 from Sri Lanka, compared with genome sequence of strain H-08-1320\*

Protein, amino acid substitution	Site/domain/region of protein†
<b>N</b>	
Leu <sub>80</sub> → Phe <sub>80</sub>	
Glu <sub>110</sub> → Asp <sub>110</sub>	
Ile <sub>246</sub> → Val <sub>246</sub>	
Ala <sub>372</sub> → Val <sub>372</sub>	Antigenic site I
<b>P</b>	
Gln <sub>167</sub> → Arg <sub>167</sub>	N protein binding site in variable domain II
<b>M</b>	
Ile <sub>16</sub> → Ala <sub>16</sub>	
Pro <sub>19</sub> → Ser <sub>19</sub>	
Ile <sub>55</sub> → Val <sub>55</sub>	
Lys <sub>77</sub> → Arg <sub>77</sub>	
<b>G</b>	
Val <sub>193</sub> → Ile <sub>193</sub>	
Arg <sub>264</sub> → His <sub>264</sub>	
Ile <sub>449</sub> → Thr <sub>449</sub>	Transmembrane region
Thr <sub>459</sub> → Ile <sub>459</sub>	Transmembrane region
Ala <sub>467</sub> → Thr <sub>467</sub>	Cytoplasmic domain
Glu <sub>475</sub> → Gly <sub>475</sub>	Cytoplasmic domain
Asn <sub>499</sub> → Ser <sub>499</sub>	Cytoplasmic domain
<b>L</b>	
Ser <sub>26</sub> → Pro <sub>26</sub>	
Ile <sub>49</sub> → Leu <sub>49</sub>	
Cys <sub>137</sub> → Tyr <sub>137</sub>	
Leu <sub>222</sub> → Ile <sub>222</sub>	
Ser <sub>312</sub> → Gln <sub>312</sub>	Conserved domain I
Glu <sub>313</sub> → Lys <sub>313</sub>	Conserved domain I
Ser <sub>314</sub> → Ala <sub>314</sub>	Conserved domain I
Arg <sub>315</sub> → Glu <sub>315</sub>	Conserved domain I
Val <sub>317</sub> → Phe <sub>317</sub>	Conserved domain I
Lys <sub>1056</sub> → Arg <sub>1056</sub>	Conserved domain IV
Thr <sub>1137</sub> → Val <sub>1137</sub>	Conserved domain V
Ala <sub>1520</sub> → Glu <sub>1520</sub>	
Ile <sub>1555</sub> → Val <sub>1555</sub>	
Leu <sub>1570</sub> → Met <sub>1570</sub>	
Met <sub>1577</sub> → Leu <sub>1577</sub>	
Lys <sub>1625</sub> → Arg <sub>1625</sub>	
Asn <sub>1763</sub> → Asp <sub>1763</sub>	
Arg <sub>1876</sub> → His <sub>1876</sub>	
Asn <sub>2023</sub> → Asp <sub>1763</sub>	
Gly <sub>2098</sub> → Arg <sub>2098</sub>	
Leu <sub>2113</sub> → Phe <sub>2113</sub>	

\*H-08-1320 is a human strain typical of canine rabies virus circulating in Sri Lanka; H-1413-09 is a novel sylvatic rabies virus variant from a golden palm civet in Sri Lanka.

†Blank spaces indicate no site/domain/region has been identified in that portion of the protein.

maximizing replication and dispersal opportunities (9). Most viruses replicate poorly when transferred to new hosts, but greater genetic variation assists in such species adaptation (10). Increased mutation in an RNA virus like rabies virus can give rise to variants with altered levels of fitness to persist and spread. A large number of substitutions were found in strain H-1413-09 compared with strain H-08-1320; these substitutions might represent changes that resulted from species adaptation. Phylogenetic analysis and comparative sequence data indicated that strain H-1413-09 is a variant rabies virus.

Palm civets are facing extinction in Sri Lanka because the species is losing its habitat, being hunted for its meat, and dying of parasitic diseases ([www.sundaytimes.lk/090118/Plus/sundaytimesplus\\_01.html](http://www.sundaytimes.lk/090118/Plus/sundaytimesplus_01.html)). Our study indicates that rabies might be another risk factor for extinction of these animals. Identification of a variant rabies virus in wildlife has serious implications for rabies control in Sri Lanka. Identification of such a virus would help provide epidemiologic data about the spread of rabies and its incursion into new geographic regions and would justify allocation of increased resources to help control rabies (11,12).

Several rabies virus variants associated with wildlife are known in the Americas and Africa (1,13–15), and this report identified classical sylvatic rabies in Asia. Whether *P. zeylonensis* is a reservoir of rabies virus or represents spillover from another animal deserves extensive investigation. The detection of rabies in wildlife indicates that much remains to be discovered in the tropical ecosystem of Sri Lanka. The circulation of a sylvatic variant rabies virus may be another hurdle in the rabies-control effort in Sri Lanka.

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